Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information for

Circular Permutation of the Trp-cage: Fold Rescue upon Addition of a Hydrophobic Staple

Aimee Byrne, Brandon L. Kier, D. V. Williams, Michele Scian, and Niels H. Andersen*

Representative CD data.

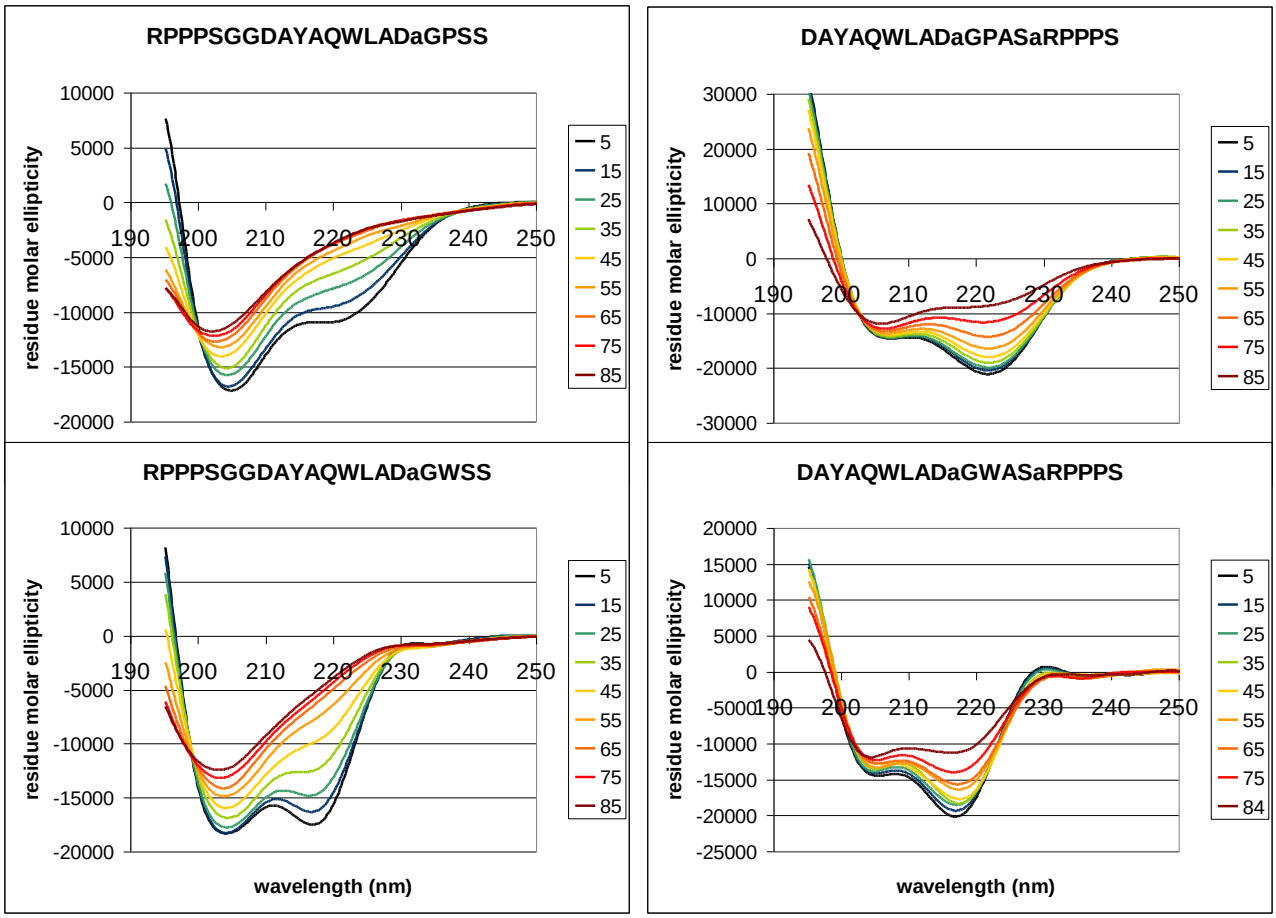

Figure 1S. Circular Dichroism Temperature Scan for cp-TC2 (upper left), cp-T^{2}C3 (lower left), and standard sequence-order controls, TC16b (upper right) and its [P12W]-mutant, T^{2}C16b (lower right). All peptides display helical signatures, but the standard topology gives rise to significantly higher melting points. A comparison of the two left panels also indicates that the P12W mutation increases the fold stability of the circular permutant.

CSD (Chemical Shift Deviation) calculations and reference compounds

- The P12W mutation does not alter shift deviations for residues 1-14,16,19-20 of the Trp-cage but some additional ring current shifts do result: P^{17}Hα (which appears at its coil value in Trp-cages) and P^{18}Hδ3 are both 0.5 ppm further upfield in the Trp^2-cage, and W^{6}Hζ2 (upfield by 1.7 ppm vs 0.2 ppm in the corresponding Trp-cage). Additional ring current effects due to W12 are also observed at sites that were shielded by the W6 indole ring. For example, the P^{18} Hα and Hβ3 resonances, which appear at 2.40 – 2.32 and 0.21 – 0.12 ppm, respectively [*5b*] in previous optimized Trp-cage species, move to 2.04 and – 0.42 ppm for this Trp^2-cage. The CSD comparisons shown in Fig. 3 reflect the appropriate non-cyclic

reference compound for each mutated permutant structure. The direct CSD comparison of cp-T^{2}C3b and (P12W)-T^{2}C16b appears below (Fig. 2S).

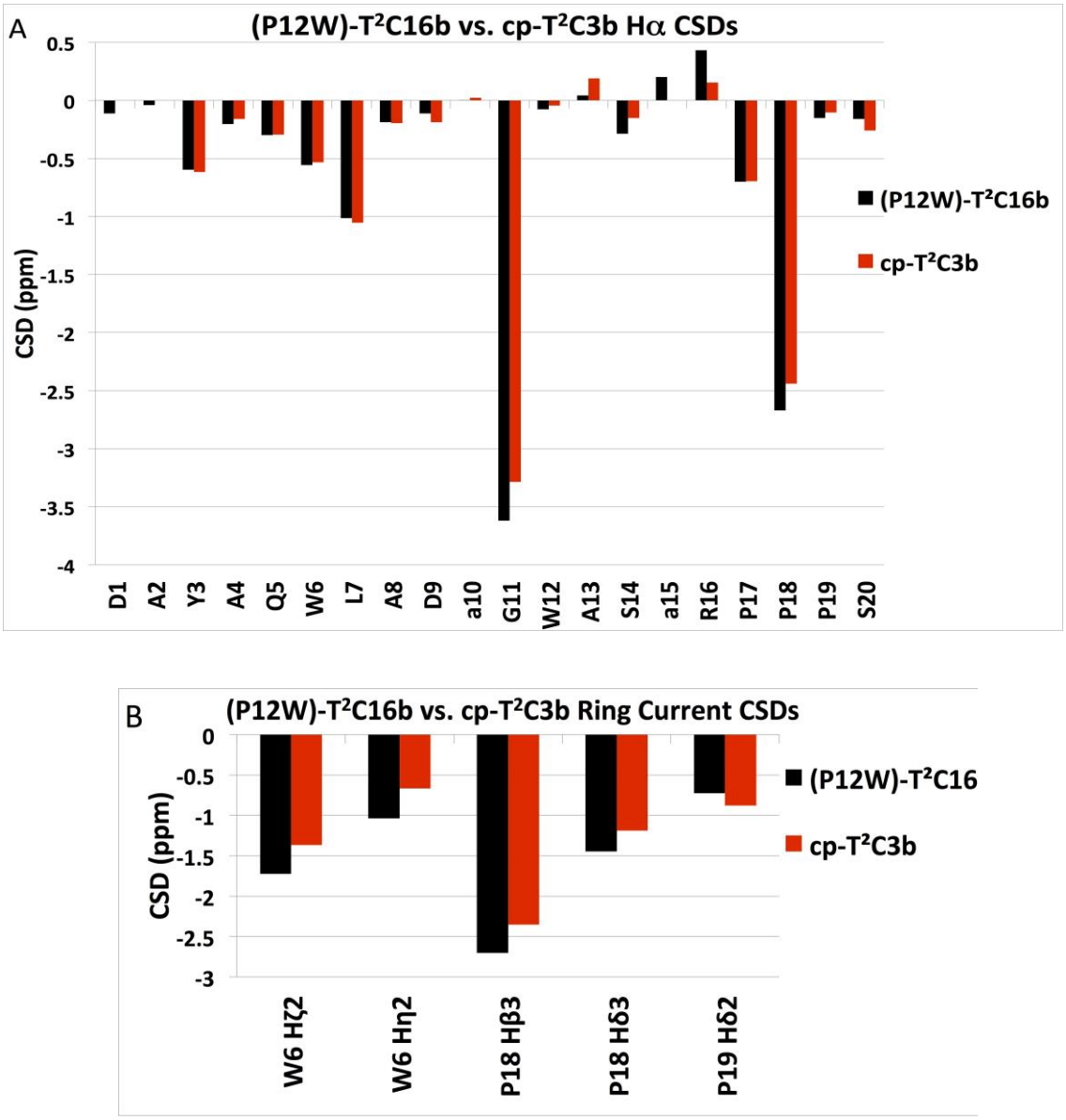

Figure 2S. CSD comparisons for (P12W)-T^{2}C16b and its circular permutant (cp-T^{2}C3b). The upfield shift of H17α seen in panel A, is not observed for species lacking the P12W mutation. The other large shifts in panel A are observed for all Trp-cage species. The large shifts in panel B are specifically associated with the added tryptophan (ring current effects**).**

The ring current shifts associated with the added Trp of the circular permutant (P19W) differ from those of (P12W)-TC16b only at a single P19 resonance. The shifts observed at P17Hα (panel A) and at other sites (panel B) are analogous to those in the normal topology reference, but equally diminished in magnitude due to a lower fold population and contributions from species with a frayed C-terminus.

Comparisons of mutational ΔΔG values, circular permutants versus normal topology.

The additional stability data upon which $\Delta\Delta G_U$ increments for key mutations in both non-permuted and permuted Trp-cages are based are collected in Table S1. CD melting temperature are included where available. The sequences of the non-permuted reference compounds are shown here: `DAYAQ WLKDG GPSSG RPPPS` (TC10b), `DAYAQ WLADG GPASG RPPPS` (TC13b), `DAYAQ WLADa GPASa RPPPS` (TC16b), and `NLYIQ WLKDG GPSSG RPPPS` (TC5b).

Similar data for cyclo-TC1 [cyclo-(`GDAYAQ WLADG GPSSG RPPPSG`)] appears here: Tm = 95 (pH = 6 -7), = 84 °C (pH = 2.5), implying an acidification $\Delta\Delta G_U$ of –2.5 kJ/mol. The Tm for the corresponding non-cyclic control, `GDAYAQ WLADG GPSSG RPPPSG` , was 58 °C. All of these were determined from CD melts.

In Table S1, instances of decreased thermal stability associated with inclusion of glycines in the loop are indicated by yellow highlighting. The corresponding reference comparisons are shown with blue highlighting.

Additional insights from loop substitutions in the circular permutants. The alternative loop insertions examined provide numerous examples in which the inclusion of glycines can be seen to decrease the thermal robustness of the fold. An Aib residue provides an additional increment of thermal robustness versus an alanine. At low temperatures the most fold-stabilizing substitution for the original SGGDA loop was SDAAL, but the SDUAA loop provided the more stable fold at tempertures greater than 35 °C. The fold stabilizing effect of Aib insertion, presumably associated with its strong preference for helical phi/psi values, is maximal when Aib is the first helical residue and remains when it is moved to the second position; however, Aib is notably destabilizing as the 3rd helical residue (ΔΔG circa 6 kJ/mol versus the optimal 1st position). Studies in designed helices indicate that this is a general feature of Aib insertions into helices.

Table 1S. Mutational Effects in the Trp-cage and its Circular Permutants

Construct	fraction folded (χ_F) at			Tm, NMR (***CD***)	ΔG_U(kJ/mol) at		ΔTm
	280 K	300 K	320 K		280 K	300 K	
cp-TC1	0.435	0.230	0.098	1	-0.609	-2.81	ref
at pH 2.5	0.20	0.12					
$\Delta\Delta G_U$					-2.61	-2.0	
cp-TC2 (= G10**a**)	0.591	0.374	0.201	17	0.857	-1.20	16
$\Delta\Delta G_U$					+1.47	+1.6	
S20A (= S13A)	0.615	0.392	0.245	20	1.09	-1.02	3
$\Delta\Delta G_U$					+0.23	+0.18	
S21A (= S14A)	0.434	0.316	0.202	4	-0.618	-1.80	-13
$\Delta\Delta G_U$					-1.47	-0.60	
P19W (= P12W)	0.786	0.586	0.336	35	3.03	0.809	18
$\Delta\Delta G_U$					+2.17	+2.0	

cp-TC2 loop mutants							
WT loop = SGGDA	0.591	0.374	0.201	17	0.857	-1.20	ref
loop = SGNAA	0.584	0.338	0.178	15	0.786	-1.56	-2
loop = SGGNAA	0.496	0.279	0.138	6	-0.038	-2.21	-11
loop = SGGGGNAA	0.586	0.284	0.124	13	0.808	-2.15	-4
loop = SPADA	0.345	0.21	0.13	< 0	-1.49	-3.08	
loop = STADA (= cp-TC2b) $\Delta\Delta G_U$	0.635	0.44	0.281	22.5(***19***)	1.29 +0.43	0.562 +0.64	3
loop = STUDA	0.62	0.495	0.37	26.5	1.14	-0.05	9
loop = SGUDA	0.756	0.578	0.362	36	2.63	0.732	19
loop = SDAAA	0.648	0.528	0.42	36.5	1.42	0.26	19.5
loop = SDAUA	0.572	0.42	0.34	14 (***18***)	0.675	-0.75	~0
loop = SDAAL $\Delta\Delta G_U$	0.791	0.635	0.462	43.5	3.10 +1.81	1.29 +2.5	26.5
loop = SDAAU	0.18	0.11	0.09	<< 0	-3.53	-4.87	
loop = DGUDA	0.78	0.58	0.366	36.5	2.95	0.75	19.5
loop = GAUDA	0.506	0.356	0.243	8	0.056	-1.38	-9
cp-TC2c, loop = SDUAA $\Delta\Delta G_U$	0.734	0.595	0.502	48(***48.5***)	2.36 +1.5	0.896 +2.1	31
(S20A)-SDUAA				(***50.4***)			2
cp-T^2C3b = (P19W,S20A)-SDUAA $\Delta\Delta G_U$	0.936	0.824	0.689	~63 (***67***)	6.24 +5.38	3.59 +4.8	48
Non-cp-controls							
TC5b at pH2.5 (P12W) [*17*]	0.98	0.92 0.45	0.43	(***42***) (***25***) (57)	9.0 (-4.3 est) (3.9 est)		-17 15
TC10b at pH2.5 (S13A) (S14A) (G10**a**)	0.99 ~0.9 0.98	0.90 0.67 0.91 0.44 0.97	0.67 0.41 0.86	57 (***56***) 39 (***41***) (***62***) 21 (***72.5***)	12.2 (-4.1 est) (1.8 est) (-8.4 est) (4.0 est)		-16 4 -35 16.5
TC13b (P12W)				(***68***) (***77***)	(2.3 est)		9
TC16b at pH2.5 (P12W)		0.99 0.95	0.95 0.85	(***83***) (***74***) (***97***)	17.9 (-2.3 est) (3.5 est)		-9 14

NMR Structure Ensemble Calculations.

Previously used procedures [*5b, 11, 22*] were employed to generate NMR ensembles for cp-T^2C3b and (P12W)-T^2C16b from the NOE intensities observed in τ_{mix} = 50 ms and 80 ms, respectively, in NOESY spectra recorded at 800 and 750 MHz, respectively. Additional long-range constraints, and data that was used to determine which constraints were influenced by secondary NOEs came from longer τ_{mix} experiments. The chemical shifts and the NOE-derived constraints appear in Tables 5S and 6S. The resulting ensembles are compared in Figure 3S, immediately below. We replaced the Aib in cp-T^2C3b with a glycine for the restrained dynamics runs (and deleted the NOE constraints for the Aib methyl groups). The two ensembles of accepted structures from CNS are shown below (Fig. 3S).

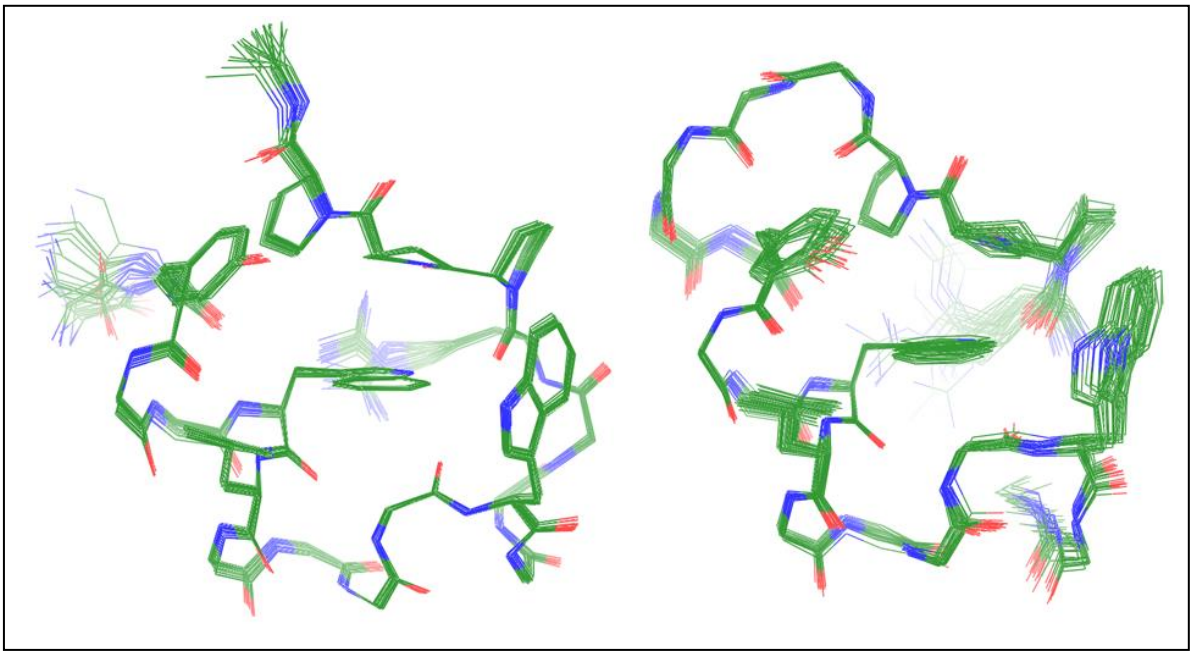

Figure 3S. (P12W)-TC16b (left panel) generated from 30/40 lowest energy structures. cp-T^2C3b (right panel) ensemble generated from 32/40 lowest energy structures. Structure and violation statistics for the ensembles appear in Tables 2S and 3S.

We reinserted the methyl goups of the Aib unit for a final steepest descent minimization to produce the structures that have been deposited at the PDB; these structures do predict the observed NOE contacts with the Aib methyl groups. Views of this ensemble and that of (P12W)-TC16b appear in Fig. 4S.

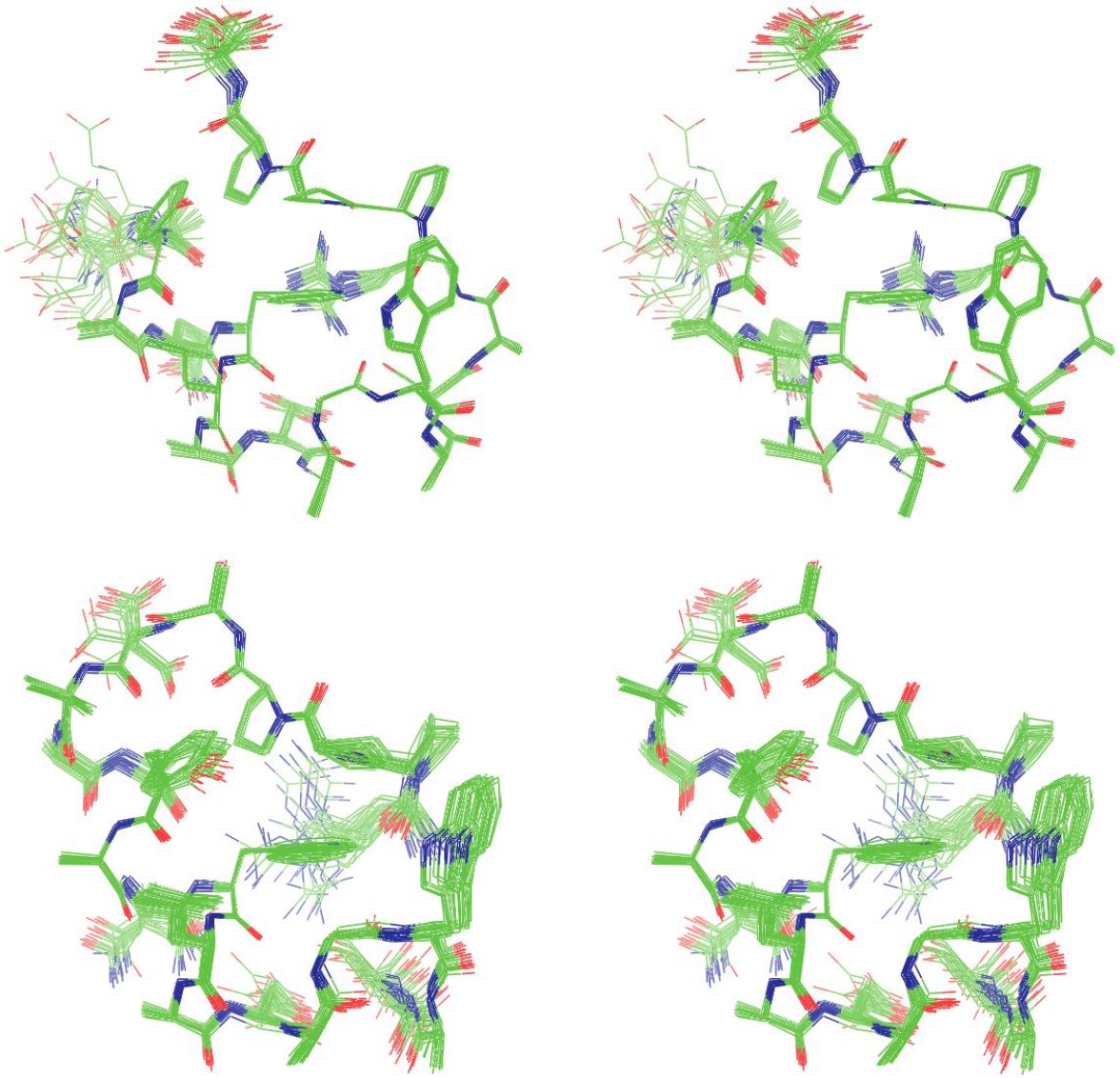

Figure 4S. Stereo views of the above ensembles; the optimized standard-topology Trp-cage (P12W)-T^2C16b (top) and the optimized circular permutant cp-T^2C3b (below).

Table 2S. NMR structure statistics for the (P12W)-T^{2}C16b ensemble (30 accepted structures from 40 starts).

Type of constraint	Number	r.m.s deviation
Intraresidue	120	0.022 ± 0.004
Sequential	80	0.025 ± 0.006
i/i+n, n = 2-4	42	0.027 ± 0.016
i/i+n, n ≥ 5	65	0.037 ± 0.002
Structure statistics[a.].		
E_{TOTAL} (kcal/mol)		-39.3 ± 1.21
E_{NOE} (kcal/mol)		19.5 ± 0.49
E_{vdW} (kcal/mol)		-84.9 ± 1.1
Bond violations (Å)		3.29 ± 0.079
Angle violations (°)		19.9 ± 0.28
Improper torsion violations (°)		2.87 ± 0.12
Convergence winthin final ensemble, atomic r.m.s deviations (Å)[b.]:		
Pairwise over the ensemble ((±s.e.)		
Backbone		0.03 ± 0.01
Heavy atom		0.36 ± 0.13

Table 3S. NMR structure statistics for the cp-T^{2}C3b ensemble (32 accepted structures from 40 starts).

Type of constraint	Number	r.m.s deviation
Intraresidue	132	0.014 ± 0.003
Sequential	73	0.051 ± 0.043
i/i+n, n = 2-4	49	0.018 ± 0.015
i/i+n, n ≥ 5	47	0.04 ± 0.064
Structure statistics[a.].		
E_{TOTAL} (kcal/mol)		-64.9 ± 4.3
E_{NOE} (kcal/mol)		4.18 ± 0.20
E_{vdW} (kcal/mol)		-90.7 ± 4.0
Bond violations (Å)		3.10 ± 0.19
Angle violations (°)		17.4 ± 0.87
Improper torsion violations (°)		1.15 ± 0.10
Convergence winthin final ensemble, atomic r.m.s deviations (Å)[b.]:		
Pairwise over the ensemble ((±s.e.)		
Backbone		0.19 ± 0.06
Heavy atom		0.65 ± 0.16

[a.] Values are mean ± standard deviation. [b.] All convergence measures are over residues 3-19.

Table 4S. Dihedral angles statistics (±s.d.) for the NMR ensembles of (P12W)-T^{2}C16b (30 structures)and cp-T^{2}C3b (32 structures), as well as the loop of cyclo-TC1.

(P12W)-T^{2}C16b				cp-T^{2}C3b			
Residue	Φ	Ψ	χ^1	Residue	Φ	Ψ	χ^1
D1		112.6(105.2)	-118.8(76.7)				
A2	-117.0(75)	-45.1(31.2)		A9	-68.9(2.0)	-29.9(0.7)	
Y3	-66.4(3)	-50.1(2.4)	-151.5(0.6)	Y10	-61.2(0.6)	-35.2(0.2)	-168.7(2.0)
A4	-65.6(1.5)	-34.2(1.3)		A11	-69.7(1.5)	-53.2(0.3)	
Q5	-69.0(1.2)	-38.4(0.6)	178.1(2.8)	Q12	-60.3(0.7)	-35.5(1.0)	-152.3(1.1)
W6	-60.7(0.6)	-48.9(0.2)	179.2(0.4)	W13	-56.4(0.9)	-46.8(1.2)	179.7(0.9)
L7	-64.4(0.4)	-36.4(0.3)	-62.4(1.1)	L14	-62.4(1.1)	-42.9(1.4)	-68.0(0.3)
A8	-75.4(0.7)	-18.3(1.0)		A15	-59.7(1.4)	-31.8(2.1)	
D9	-88.8(1.0)	15.7(0.6)	-117.9(9.6)	D16	-87.3(3.7)	10.9(8.6)	-129.4(12.3)
a10	103.3(0.8)	-3.4(0.4)		a17	90.3(10.6)	7.4(9.1)	
G11	67.3(0.2)	-96.9(0.4)		G18	61.7(3.4)	-105.6(3.4)	
W12	-97.3(0.4)	10.8(1.6)	-82.0(0.3)	W19	-68.2(2.7)	-30.0(1.8)	-74.4(4.1)
A13	-85.5(1.6)	-31.8(0.4)		A20	-90.4(8.0)	36.8(17.4)	
S14	-62.9(0.7)	-29.6(0.7)	32.7(0.9)	S21	-93.4(12.5)		178.8(30.2)
a15	100.1(0.7)	-24.3(0.5)	89.3(86.5)				
R16	-86.0(0.4)	153.6(0.3)	80.1(1.1)	R1		133.6(8.6)	57.9(6.9)
P17	-83.5(0.4)	166.1(0.5)	5.0(0.4)	P2	-73.9(10.3)	164.0(5.4)	8.0(23.1)
P18	-62.8(0.8)	145.9(0.9)	17.8(0.5)	P3	-53.4(3.9)	117.3(3.7)	1.4(17.3)
P19	-80.8(0.4)	142.2(12)	22.7(0.4)	P4	-81.5(1.4)	142.4(6.6)	30.7(0.6)
S20	-129.9(30)		57.2(80.2)				
cyclo-TC1							
S20	-86(7)	-31(6)		S5	-57.7(4.9)	-44.9(2.8)	52.8(1.1)
G21	-74(8)	147(4)		D6	-57(6)	142(7)	-55(78)
G(-1)	-67(4)	-40(6)		U7	-70(7)	-38.3(1.5)	
D1	-70.0(4.0)	-43.0(3.0)		A8	-56.4(2.8)	-56.9(1.1)	

Additional dihedral angle comparisons for Trp-cages are given in M. Scian *et al.* [*11*]; these include the averages for all Trp-cages prior to the two additions shown above.

Table 5S. Chemical shifts and the NOE constraint list for (P12W)-T[2]C16b

Chemical shifts at 280K

#	Res		Hα	Hβ	Hγ	Hδ	Hε	Hζ	Hη
1	Asp		4.252	3.153, 2.962	-----		-----	-----	-----
2	Ala	8.311	4.246	1.488	-----	-----	-----	-----	-----
3	Tyr	8.849	3.952	3.185[2], 3.529[3]	-----	7.053	6.780	-----	
4	Ala	8.311	4.083	1.572	-----	-----	-----	-----	-----
5	Gln	8.129	3.950	2.196, 2.121	2.395	-----	7.881, 7.048	-----	-----
6	Trp	8.033	4.096	3.033[2], 3.444[3]	-----	6.764	9.600, 6.814	5.732, 6.876	6.160
7	Leu	8.381	3.284	1.837[2], 1.338[3]	1.569	0.968[1], 0.858[2]	-----	-----	-----
8	Ala	8.161	4.000	1.468	-----	-----	-----	-----	-----
9	Asp	7.953	4.531	2.660[2], 2.764[3]	-----		-----	-----	-----
10	dAla	7.378	4.309	1.263	-----	-----	-----	-----	-----
11	Gly	8.393	0.309[2], 3.013[3]	-----	-----	-----	-----	-----	-----
12	Trp	9.118	4.596	3.528, 3.182	-----	7.628	10.583, 7.924	7.620, 7.213	7.340
13	Ala	7.806	4.351	1.482	-----	-----	-----	-----	-----
14	Ser	8.047	4.080	3.840		-----	-----	-----	-----
15	dAla	7.452	4.519	1.613	-----	-----	-----	-----	-----
16	Arg	8.143	5.046	1.859, 1.733	1.729, 1.540	3.260, 3.156	7.651	-----	
17	Pro	-----	3.999	1.927[2], 2.379[3]	2.065, 1.739	3.622[2], 3.826[3]	-----	-----	-----
18	Pro	-----	2.030	1.096[2], -0.421[3]	1.543, 1.247	3.182, 2.290	-----	-----	-----
19	Pro	-----	4.289	1.960[2], 2.167[3]	1.793, 1.735	2.863[2], 3.097[3]	-----	-----	-----
20	Ser	7.938	4.140	3.772		-----	-----	-----	-----

Distance constraints employed in genrating the NMR structure ensemble.

Residue #	Atom Name	Shift (ppm)	Residue #	Atom Name	Shift (ppm)	d (Å)	d– (Å)	d+ (Å)
Intraresidue								
1	ha	4.21	1	hb2	3.11	2.80	0.72	0.66
1	hb1	2.95	1	ha	4.22	3.00	0.85	0.89
2	ha	4.25	2	hb#	1.49	2.50	0.27	0.24
3	hb2	3.08	3	hd1	7.04	2.41	0.21	0.51
3	hb1	3.14	3	hd2	7.04	2.47	0.26	0.53
3	ha	3.97	3	hb2	3.08	2.63	0.32	0.26
3	ha	3.97	3	hd1	7.04	3.20	0.34	0.48
3	ha	3.97	3	hb1	3.13	3.09	0.46	0.37
3	hb2	3.08	3	hn	8.75	2.90	0.57	0.47
3	hb1	3.13	3	hn	8.75	3.58	0.71	0.66
3	ha	3.97	3	hn	8.75	2.90	0.90	1.00
4	ha	4.08	4	hb#	1.57	2.35	0.23	0.22
4	hb#	1.57	4	hn	8.29	2.44	0.25	0.23
4	ha	4.07	4	hn	8.29	2.80	0.37	0.30
5	hg1	2.40	5	hb1	2.19	2.54	0.29	0.25
5	hb*	2.13	5	hn	8.04	2.69	0.34	0.27
5	ha	3.96	5	hn	8.04	2.76	0.36	0.29
5	hg#	2.40	5	he22	7.80	2.82	0.38	0.90
5	ha	3.95	5	hg1	2.40	2.83	0.38	0.30

5	ha	3.96	5	hb2	2.19	2.60	0.35	0.36
5	hg1	2.40	5	hn	8.04	3.53	0.48	0.78
5	hg2	2.40	5	he21	6.94	2.75	0.51	0.71
5	ha	3.96	5	he22	7.80	4.16	0.80	0.80
6	hb1	3.44	6	hn	7.99	2.47	0.26	0.23
6	hb2	3.04	6	hn	7.99	2.59	0.30	0.26
6	hb1	3.44	6	he3	6.82	2.68	0.33	0.27
6	hb2	3.03	6	hd1	6.77	2.68	0.33	0.27
6	ha	4.11	6	hd1	6.76	2.75	0.35	0.29
6	ha	4.11	6	hn	7.99	2.89	0.40	0.32
6	hb1	3.44	6	hd1	6.76	3.00	0.43	0.85
6	ha		6	hb2		2.50	0.10	0.10
6	ha		6	hb1		3.10	0.20	0.20
6	hb2	3.04	6	he3	6.82	3.11	0.47	0.87
6	ha	4.09	6	he3	6.82	4.30	0.85	0.90
7	hg	1.57	7	hd1#	0.95	2.60	0.35	0.54
7	hg	1.57	7	hd2#	0.85	2.80	0.45	0.64
7	hg	1.57	7	hn	8.35	2.50	0.27	0.24
7	hb2	1.82	7	hn	8.35	2.63	0.32	0.26
7	hb2	1.82	7	hd1#	0.95	2.66	0.32	0.57
7	hb1	1.34	7	hd1#	0.95	2.68	0.33	0.57
7	ha	3.28	7	hn	8.35	2.70	0.34	0.28
7	ha	3.28	7	hd2#	0.85	2.77	0.36	0.99
7	hb1	1.34	7	hn	8.35	2.85	0.39	0.95
7	hb1	1.34	7	hd2#	0.85	2.88	0.39	0.62
7	ha	3.28	7	hb1	1.33	2.50	0.35	0.36
7	ha	3.29	7	hb2	1.82	3.17	0.49	0.39
7	hb2	1.82	7	hd2#	0.85	3.27	0.52	0.72
7	hd1#	0.95	7	hn	8.35	3.35	0.54	0.74
7	ha	3.28	7	hd1#	0.95	3.38	0.56	1.50
7	ha	3.27	7	hg	1.57	3.40	0.56	0.46
7	hg	1.57	7	hb1	1.33	3.57	0.61	0.52
8	ha	4.00	8	hb	1.47	2.15	0.17	0.19
8	hb	1.47	8	hn	8.10	2.22	0.19	0.20
8	ha	4.00	8	hn	8.10	2.62	0.31	0.26
9	hb2	2.69	9	hn	7.88	2.54	0.29	0.25
9	ha	4.48	9	hn	7.88	2.84	0.38	0.31
9	ha		9	hb1		3.20	0.38	0.31
9	hb1	2.82	9	hn	7.88	2.85	0.39	0.91
10	ha	4.30	10	hb#	1.26	2.21	0.18	0.35
10	hb#	1.26	10	hn	7.36	2.43	0.25	0.73
10	ha	4.30	10	hn	7.36	3.12	0.47	0.38
11	ha2	0.33	11	hn	8.33	2.49	0.27	0.24
11	ha1	3.00	11	hn	8.33	2.64	0.32	0.26
12	hb2	3.18	12	hd1	7.62	2.36	0.23	0.82
12	ha	4.59	12	he3	7.92	2.62	0.31	0.76
12	hb1	3.54	12	he3	7.92	2.76	0.36	0.29
12	hb2	3.18	12	hn	8.98	2.50	0.44	0.35
12	ha	4.59	12	hn	8.98	2.92	0.54	0.44
12	hd1	7.62	12	hn	8.98	3.60	0.62	0.54
12	hb2	3.18	12	hz3	7.21	4.09	0.78	0.77
12	ha		12	hb2		3.10	0.10	0.10
12	ha		12	hb1		2.50	0.20	0.20
12	hb1	3.54	12	hn	8.98	3.26	0.52	0.42
12	hb2	3.53	12	hd1	7.62	2.69	0.14	0.80

12	hb1	3.18	12	he3	7.92	3.16	0.18	0.99
13	hb#	1.48	13	hn	7.78	2.16	0.16	0.91
13	ha	4.34	13	hb#	1.47	2.23	0.19	0.20
13	ha	4.34	13	hn	7.78	2.69	0.33	0.27
14	ha	4.09	14	hb2	3.45	2.59	0.30	0.26
14	ha	4.09	14	hb1	3.87	2.67	0.33	0.27
14	ha	4.09	14	hn	8.00	2.73	0.35	0.28
14	hb1	3.86	14	hn	8.00	3.09	0.46	0.37
14	ha	4.08	14	hg	4.29	4.01	0.75	0.72
14	hg		14	hb2		2.75	0.35	0.42
14	hb1		14	hg		2.30	0.26	0.32
15	hb#	1.61	15	hn	7.43	2.22	0.19	0.70
15	ha	4.50	15	hb#	1.61	2.24	0.19	0.20
16	hb1	1.84	16	hn	8.10	2.63	0.35	0.33
16	hg2	1.83	16	hn	8.10	2.70	0.45	0.63
16	ha	5.03	16	hb1	1.84	3.00	0.15	0.25
16	ha	5.03	16	hb2	1.84	2.40	0.10	0.25
16	hd2	3.26	16	hg1	1.84	2.66	0.36	0.29
16	hd1	3.17	16	hg2	1.55	2.49	0.27	0.60
16	hd1	3.18	16	hb1	1.74	2.68	0.39	0.32
16	ha	5.03	16	hn	8.10	2.95	0.42	0.33
16	hd2	3.26	16	hb2	1.74	2.90	0.31	0.55
16	hg2	1.56	16	hn	8.10	2.70	0.27	0.47
16	hd1	3.17	16	hg2	1.84	2.79	0.40	0.32
16	hd2	3.26	16	hg1	1.55	2.60	0.29	0.30
16	hh1#		16	hg1		2.20	0.20	0.35
16	hh1#		16	hd1		3.60	0.40	0.60
16	hh1#		16	hd2		2.20	0.20	0.50
17	ha	4.02	17	hb1	2.37	2.30	0.37	0.30
17	ha	4.01	17	hb2	1.75	3.00	0.46	0.37
17	hd2	3.62	17	hb2	1.75	3.00	0.20	0.21
17	ha	4.02	17	hg2	2.06	4.05	0.77	0.74
17	hd1	3.82	17	hb1	2.37	4.00	0.23	0.26
18	ha	2.06	18	hb2	1.10	2.80	0.22	0.34
18	hd1	2.34	18	hg1	1.22	2.34	0.24	0.25
18	ha	2.06	18	hb1	-0.36	2.30	0.45	0.36
18	hg1	1.22	18	hb2	1.10	3.01	0.37	0.37
18	hg1	1.23	18	hb1	-0.36	2.32	0.37	0.48
18	ha	2.06	18	hd2	3.18	3.88	0.71	0.66
18	hd2	3.18	18	hb2	1.09	3.95	0.24	0.29
18	hd1	2.34	18	hb1	-0.34	3.00	0.17	0.24
18	ha	2.06	18	hg1	1.23	4.55	0.92	1.07
18	hd1	2.34	18	hb2	1.10	4.00	0.75	0.72
18	hg2	1.55	18	hb2	1.10	2.50	0.47	0.38
19	ha	4.28	19	hb1	1.95	2.30	0.49	0.40
19	hb1	1.96	19	hg1	1.78	2.30	0.27	0.47
19	hd2	2.85	19	hg2	1.73	2.30	0.27	0.47
19	hb2	2.14	19	hg2	1.73	2.31	0.29	0.42
19	hd1		19	hb1		3.00	0.26	0.23
19	hd1	3.08	19	hb2	2.15	4.46	0.90	1.01
20	ha	4.15	20	hb2	3.76	2.50	0.37	0.37
20	ha	4.14	20	hn	7.81	3.29	0.52	0.43
20	hb#	3.77	20	hn	7.81	3.36	0.55	0.45
i, i±1								
3	hb1	3.13	4	hn	8.29	3.01	0.64	0.35

3	hb2	3.08	4	hn	8.28	3.16	0.48	0.39
3	ha	3.96	4	hn	8.28	3.56	0.61	0.52
4	ha	4.08	3	hd2	7.04	3.52	0.60	0.50
4	hn	8.29	3	hd2	7.04	3.57	0.61	0.52
4	hn	8.29	3	hn	8.75	3.42	0.66	0.59
4	hb#	1.57	5	hn	8.04	2.96	0.42	0.34
4	hn	8.29	5	hn	8.04	2.97	0.42	0.34
4	ha	4.08	5	hn	8.04	3.91	0.72	0.67
5	ha	3.96	4	hb#	1.57	4.05	0.77	0.74
5	ha	3.96	6	hn	7.99	3.56	0.25	0.36
5	hb1	2.12	6	hn	7.99	2.60	0.31	0.41
5	hg#	2.40	6	hn	7.99	4.11	0.78	0.77
6	hn	7.99	7	hn	8.35	2.68	0.33	0.27
6	hb1	3.44	7	hn	8.35	2.86	0.39	0.31
6	hb2	3.04	7	hn	8.35	3.30	0.53	0.43
7	hg	1.57	6	he3	6.82	2.17	0.33	0.37
7	hd2#	0.85	6	he3	6.82	2.70	0.34	0.98
7	hd2#	0.85	6	hz3	6.86	2.72	0.34	0.58
7	hn	8.35	6	he3	6.82	3.00	0.43	0.35
7	ha	3.28	6	he3	6.82	3.40	0.56	0.46
7	hd1#	0.95	6	he3	6.82	3.00	0.49	1.20
7	hg	1.57	6	hz3	6.86	3.66	0.64	0.56
7	hb2	1.82	6	he3	6.82	3.78	0.68	0.61
7	hd2#	0.85	6	hh2	6.14	3.63	0.70	0.93
7	hd1#	0.95	6	hz3	6.85	4.19	0.81	1.12
7	hb1	1.34	6	he3	6.82	4.41	0.88	0.97
7	hb1	1.34	8	hn	8.10	3.30	0.53	0.43
7	ha	3.27	8	hn	8.10	3.48	0.58	0.49
7	hd1#	0.95	8	hn	8.10	4.12	0.79	1.20
8	hn	8.10	7	hn	8.35	2.67	0.33	0.27
8	hn	8.11	9	hn	7.88	2.71	0.34	0.28
8	hb	1.47	9	hn	7.88	2.74	0.35	0.29
8	ha	4.00	9	hn	7.88	3.56	0.61	0.52
9	ha	4.49	8	hb	1.47	4.11	0.78	0.77
9	ha	4.49	10	hn	7.35	3.03	0.60	0.51
9	hb2	2.70	10	hn	7.36	3.93	0.73	0.68
9	hb1	2.82	10	hn	7.36	4.20	0.81	0.83
10	hn	7.36	9	hn	7.88	2.70	0.34	0.28
10	hn	7.36	11	hn	8.33	2.31	0.21	0.21
10	hb#	1.26	11	hn	8.33	3.06	0.45	0.86
10	ha	4.29	11	hn	8.33	3.34	0.54	0.44
10	hb#	1.26	14	hn	8.00	4.50	0.91	1.53
11	ha2	0.33	10	hn	7.35	3.95	0.73	0.69
11	ha1	2.99	10	hn	7.35	4.14	0.79	0.79
11	ha1	2.99	12	hn	8.98	2.60	0.38	0.31
11	ha2	0.33	12	hn	8.98	3.12	0.47	0.38
11	ha1	2.99	12	hd1	7.62	4.36	0.86	0.93
12	ha	4.59	11	ha2	0.33	4.41	0.88	0.97
12	hb2	3.18	13	hn	7.78	3.51	0.60	0.80
12	ha	4.59	13	hn	7.78	3.56	0.61	0.52
12	hb1	3.53	13	hn	7.78	3.73	0.67	0.59
13	hn	7.78	12	hn	8.98	2.70	0.27	0.25
13	hn	7.78	14	hn	8.00	2.55	0.49	0.25
13	hb#	1.47	14	hn	8.00	3.33	0.54	1.50
13	ha	4.34	14	hn	8.00	3.37	0.55	0.45

14	ha	4.09	15	hn	7.43	3.35	0.55	0.45
14	hb1	3.87	15	hn	7.43	4.12	0.79	0.78
14	hb2	3.46	15	hn	7.43	4.22	0.82	0.84
15	hn	7.43	14	hn	8.00	2.58	0.30	0.25
15	hb#	1.61	16	hn	8.09	3.03	0.44	1.25
15	ha	4.50	16	hn	8.10	3.43	0.57	0.47
16	hn	8.09	15	hn	7.43	2.45	0.26	0.23
16	hg2	1.54	15	hn	7.43	3.80	0.59	0.49
16	hg2	1.84	15	hn	7.44	4.20	0.60	0.75
16	ha	5.03	17	hd1	3.82	2.30	0.31	0.21
16	ha	5.03	17	hd2	3.62	2.79	0.27	0.24
16	hb2	1.84	17	hd2	3.62	2.95	0.38	0.31
16	hg1	1.84	17	hd2	3.62	3.60	0.39	0.31
17	hd1	3.82	16	hn	8.10	4.05	0.77	0.75
17	hd2	3.62	16	hn	8.09	4.42	0.88	0.97
17	ha	4.02	18	hd1	2.34	2.46	0.26	0.23
17	ha	4.02	18	hd2	3.18	2.55	0.29	0.25
19	hd1	3.08	18	ha	2.06	2.18	0.24	0.22
19	hd2	2.85	18	ha	2.06	2.50	0.27	0.24
19	hd2	2.85	18	hb2	1.09	3.39	0.56	0.46
19	hd1	3.08	18	hb2	1.09	3.73	0.66	0.59
19	hd2	2.85	18	hb1	-0.35	3.94	0.73	0.69
19	hd1	3.09	18	hb1	-0.36	4.09	0.78	0.77
19	ha	4.28	20	hn	7.81	2.21	0.18	0.19
19	hb1	1.96	20	hn	7.81	3.54	0.61	0.51
19	hb2	2.14	20	hn	7.81	3.71	0.66	0.58

i, i±n, n= 2-4

2	ha	4.25	5	hb1	2.12	3.00	0.59	0.50
2	ha	4.24	5	hn	8.04	3.52	0.60	0.50
2	hb#	1.47	5	hn	8.04	3.80	0.69	1.50
2	ha	4.24	5	hg1	2.40	4.09	0.78	0.76
3	ha	3.97	6	hb1	3.44	3.47	0.58	0.49
3	ha	3.97	6	hb2	3.04	3.60	0.62	0.53
3	ha	3.97	6	he3	6.82	3.73	0.67	0.59
3	ha	3.96	7	hn	8.35	3.66	0.64	0.56
4	ha	4.08	7	hd1#	0.95	2.84	0.38	0.61
4	ha	4.08	7	hn	8.35	3.20	0.50	0.40
4	ha	4.08	7	hb2	1.83	3.28	0.52	0.42
4	ha	4.08	7	hb1	1.34	3.62	0.63	0.94
5	ha	3.95	8	hb	1.47	2.52	0.28	0.24
5	ha	3.96	8	hn	8.10	3.21	0.50	0.40
5	ha	3.95	9	hn	7.88	4.18	0.81	0.82
6	ha	4.11	9	hb2	2.69	3.10	0.48	0.35
6	ha	4.10	9	hb1	2.83	3.50	0.33	0.43
6	ha	4.11	9	hn	7.88	3.48	0.59	0.49
7	hd1#	0.95	3	he2	6.77	2.95	0.42	1.20
7	hd2#	0.85	3	he2	6.77	3.06	0.45	1.20
7	hd1#	0.95	3	hd2	7.04	3.14	0.48	1.20
7	hd2#	0.86	3	hd2	7.04	4.03	0.76	1.20
7	ha	3.28	10	hn	7.35	3.67	0.65	0.56
7	ha	3.28	11	hn	8.32	2.90	0.40	0.32
7	ha	3.28	11	ha2	0.33	3.02	0.66	0.28
7	ha	3.28	11	ha1	2.99	3.89	0.72	0.66
7	hd2#	0.85	11	ha2	0.34	4.00	0.95	1.20
8	hb	1.47	5	he21	6.94	3.78	0.68	0.61

8	ha	4.01	10	hn	7.35	3.59	0.62	0.53
8	hb	1.47	10	hn	7.36	3.95	0.74	0.69
9	hb2	2.69	6	hd1	6.76	4.72	0.58	0.67
9	hn	7.88	11	hn	8.34	3.58	0.62	0.53
9	hb1	2.82	11	hn	8.33	4.12	0.45	0.65
9	hb2	2.70	11	hn	8.33	3.90	0.47	0.65
10	ha	4.30	14	hn	8.00	3.60	0.37	0.50
10	hb#	1.26	14	hn	8.00	4.50	0.91	1.53
11	ha1	3.00	7	hd2#	0.85	3.67	0.65	1.50
11	ha1	2.99	13	hn	7.78	3.99	0.75	0.71
12	ha	4.59	14	hn	8.00	3.63	0.63	0.55
12	ha	4.60	15	hn	7.43	3.92	0.72	0.68
13	ha	4.34	15	hn	7.43	3.38	0.55	0.46
14	ha	4.08	16	hn	8.10	3.52	0.32	0.73
14	hb1	3.87	16	hn	8.10	4.25	0.83	0.86
15	hb#	1.61	12	he3	7.92	3.41	0.56	0.46
15	ha	4.50	12	he3	7.91	4.37	0.87	0.94

i, i±n, n ≥ 5

3	ha	3.97	19	hd2	2.85	3.06	0.58	0.48
3	ha	3.97	19	hg2	1.73	2.70	0.51	0.52
6	hh2	6.14	12	hz2	7.62	2.65	0.39	0.71
6	hz2	5.76	12	hz2	7.62	3.80	0.42	0.93
6	hz2	5.76	12	he3	7.92	3.54	0.70	0.64
6	hh2	6.15	12	hn	8.98	4.05	0.83	0.86
6	he1	9.57	16	hn	8.10	3.64	0.64	0.55
6	hd1	6.77	16	hn	8.10	4.44	0.89	0.99
6	ha		16	hh2#		2.60	0.50	0.60
6	he1		16	O		2.00	0.10	0.20
6	ne1		16	C		4.00	0.25	0.35
9	hb1		16	hh1#		3.80	0.60	0.80
12	ha	4.60	6	he1	9.57	3.50	0.38	0.77
12	ha	4.59	6	hh2	6.14	3.95	0.80	0.80
14	hb1	3.87	9	hb1	2.81	3.47	0.58	0.49
14	hb2	3.45	9	hb1	2.81	3.66	0.64	0.56
14	hb1	3.86	9	hb2	2.69	3.93	0.73	0.68
14	hb2	3.45	9	hb2	2.69	4.00	0.75	0.72
16	hh2#		5	hg1		3.40	0.30	0.40
16	hg1	1.55	6	hd1	6.77	2.63	0.25	0.33
16	hg1	1.84	6	he1	9.57	3.15	0.38	0.48
16	hd1	3.15	6	hd1	6.77	3.46	0.58	0.48
16	hd2	3.27	6	hd1	6.76	4.23	0.82	0.85
16	hg2	1.84	6	hd1	6.76	2.55	0.29	0.25
16	he		6	hd1		3.19	0.49	0.40
17	ha	4.02	6	hz2	5.76	3.39	0.65	0.57
17	ha	4.02	6	he1	9.57	3.36	0.34	0.60
17	hb1	2.35	12	hh2	7.34	2.10	0.54	0.44
17	hb1	2.35	12	hz2	7.62	3.43	0.57	0.47
17	ha	4.02	12	hz3	7.21	3.52	0.60	0.50
17	ha	4.02	12	he3	7.92	3.77	0.68	0.61
17	hb1	2.35	12	hz3	7.21	3.77	0.68	0.61
17	hb2	1.74	12	hh2	7.34	4.19	0.81	0.83
17	hb2	1.75	12	hz3	7.21	4.24	0.83	0.85
17	ha	4.01	12	hz2	7.62	4.33	0.86	0.91
18	hb2	1.10	3	he1	6.77	3.24	0.51	0.41
18	hb1	-0.35	3	he1	6.77	3.30	0.53	0.43

18	hb2	1.10	3	hd1	7.04	3.62	0.63	0.54
18	hb1	-0.35	3	hd1	7.04	3.95	0.74	0.69
18	hg1	1.22	3	he1	6.77	4.20	0.81	0.83
18	hd1	2.34	6	hz2	5.76	2.49	0.37	0.30
18	ha	2.06	6	hd1	6.77	3.23	0.47	0.78
18	ha	2.06	6	he1	9.57	3.74	0.67	0.59
18	hd1	2.34	6	hh2	6.14	3.83	0.76	0.73
18	hg1	1.23	6	hh2	6.14	3.89	0.78	0.76
18	hb1	-0.35	6	hh2	6.13	3.93	0.79	0.79
18	hb1	-0.35	6	hz3	6.86	4.13	0.79	0.79
18	hd1	2.35	6	he1	9.57	4.23	0.32	0.45
18	hb1	-0.35	6	hz2	5.76	3.99	0.84	0.88
18	hb1	-0.35	6	he3	6.82	4.37	0.87	0.94
18	ha	2.06	6	he3	6.82	4.42	0.88	0.97
18	hg1	1.22	6	hz2	5.76	3.88	0.81	0.82
18	hg1	1.22	12	hz2	7.62	3.96	0.74	0.70
19	hb1	1.96	2	hb#	1.49	3.82	0.69	0.63
19	hb2	2.15	2	hb#	1.49	3.85	0.70	0.64
19	hd2	2.85	3	hd1	7.04	2.98	0.43	0.34
19	hd2	2.84	3	he1	6.77	3.18	0.49	0.39
19	hg2	1.79	3	hd1	7.04	3.51	0.60	0.50
19	hb1	1.96	3	hd1	7.04	3.70	0.57	0.94

Table 6S. Chemical shifts and the NOE constraint list for cp-T^{2}C3b

Chemical shifts at 280K

#	Res		Hα	Hβ	Hγ	Hδ	Hε	Hζ	Hη
1	Arg		4.509	1.979, 1.821	1.936, 1.645	3.173	7.421	-----	
2	Pro	-----	4.006	1.724, 2.422	2.101, 2.007	3.526, 3.811	-----	-----	-----
3	Pro	-----	2.262	1.12, -0.07	1.454, 1.646	3.237, 2.548	-----	-----	-----
4	Pro	-----	4.336	1.956, 2.164	1.794, 1.735	2.711, 3.054	-----	-----	-----
5	Ser	8.229	4.189	3.837, 3.764		-----	-----	-----	-----
6	Asp	7.852	4.511	2.716, 2.618	-----		-----	-----	-----
7	Aib	8.769	-----	1.419	-----	-----	-----	-----	-----
8	Ala	8.338	4.239	1.500	-----	-----	-----	-----	-----
9	Ala	8.272	4.474	1.542	-----	-----	-----	-----	-----
10	Tyr	8.545	3.931	3.183, 3.119	-----	7.011	6.754	-----	
11	Ala	8.417	4.125	1.585	-----	-----	-----	-----	-----
12	Gln	8.164	3.955	2.181, 2.10	2.407	-----	8.027, 7.048	-----	-----
13	Trp	7.964	4.120	2.997, 3.515	-----	6.752	9.214, 6.817	6.093, 6.876	6.530
14	Leu	8.458	3.245	1.725, 1.433	1.512	0.940, 0.880	-----	-----	-----
15	Ala	7.924	3.991	1.466	-----	-----	-----	-----	-----
16	Asp	8.099	4.454	2.711, 2.801	-----		-----	-----	-----
17	dAla	7.290	4.328	1.225	-----	-----	-----	-----	-----
18	Gly	8.187	0.645, 2.807	-----	-----	-----	-----	-----	-----
19	Trp	8.767	4.627	3.517, 3.166	-----	7.517	10.53, 7.849	7.584, 7.243	7.345
20	Ala	7.851	4.498	1.406		-----	-----	-----	-----
21	Ser	7.580	4.047	3.469, 3.861		-----	-----	-----	-----

Distance constraints employed in genrating the NMR structure ensemble.

Residue #	Atom Name	Shift (ppm)	Residue #	Atom Name	Shift (ppm)	d (Å)	d– (Å)	d+ (Å)
Intraresidue (i,i) constraints								
10	he#	6.76	10	hd#	7.01	1.96	0.06	1.02
10	hb2	3.11	10	hd#	7	2.48	0.25	0.65
10	hb2	3.12	10	hn	8.54	2.56	0.29	0.25
10	hb1	3.19	10	hn	8.54	2.7	0.34	0.28
10	hb1	3.18	10	hd#	7	2.71	0.32	0.69
10	ha	3.94	10	hb1	3.2	2.98	0.43	0.34
10	ha	3.94	10	hb2	3.13	2.71	0.37	0.32
10	ha	3.94	10	hd1	7	3.12	0.45	0.78
10	ha	3.94	10	hn	8.54	3.18	0.49	0.39
11	ha	4.14	11	hb#	1.6	2.53	0.18	0.22
11	hb#	1.59	11	hn	8.41	2.86	0.29	0.26
11	ha	4.13	11	hn	8.41	2.87	0.39	0.31
12	hg#	2.4	12	hb2	2.18	2.46	0.2	0.2
12	hg#	2.41	12	he#	8.01	2.39	0.21	0.35
12	ha	3.96	12	hb1	2.1	2.78	0.25	0.22
12	ha	3.96	12	hn	8.15	2.64	0.26	0.23
12	hb2	2.19	12	hn	8.15	2.52	0.28	0.24
12	hb1	2.1	12	hn	8.15	2.62	0.31	0.26
12	ha	3.97	12	hb2	2.19	2.71	0.34	0.28
12	ha	3.96	12	hg#	2.4	2.84	0.38	0.31
12	ha	3.96	12	he#	8.01	3.54	0.6	0.51
12	hg3	2.4	12	hn	8.15	3.78	0.68	0.61
13	hz3	6.88	13	he3	6.82	2.2	0.2	0.54
13	hb1	3.52	13	hn	7.96	2.33	0.22	0.21
13	hh2	6.54	13	hz3	6.88	2.41	0.25	0.62
13	hb2	3	13	hn	7.96	2.5	0.27	0.24
13	hz2	6.1	13	hh2	6.53	2.5	0.27	0.64
13	hd1	6.76	13	he1	9.21	2.6	0.31	0.66
13	ha	4.12	13	hn	7.95	2.65	0.32	0.27
13	hb2	3	13	hd1	6.75	2.66	0.32	0.47
13	hb1	3.52	13	he3	6.81	2.78	0.36	0.49
13	ha	4.12	13	hb2	3	2.82	0.38	0.3
13	ha	4.12	13	hd1	6.75	2.92	0.41	0.53
13	ha	4.14	13	hb1	3.54	3.04	0.45	0.36
13	hz2	6.1	13	he1	9.21	3.11	0.47	0.77
13	hb2	3	13	he3	6.81	3.73	0.66	0.79
13	hb1	3.52	13	hd1	6.75	3.81	0.69	0.82
14	hb2	1.73	14	hn	8.45	2.64	0.25	0.23
14	hg	1.52	14	hn	8.45	2.55	0.29	0.25
14	ha	3.25	14	hn	8.45	2.69	0.33	0.27
14	ha	3.25	14	hb1	1.44	2.51	0.34	0.28
14	hb1	1.44	14	hn	8.45	3.31	0.37	0.3
14	ha	3.24	14	hb2	1.71	3.08	0.39	0.31
14	ha	3.25	14	hd2#	0.88	2.74	0.37	0.31
14	ha	3.25	14	hg	1.51	3.39	0.62	0.53
14	hb2	1.73	14	hd2#	0.88	3.72	0.56	0.45
14	ha	3.25	14	hd1#	0.93	4.09	0.58	0.67
14	hd2#	0.88	14	hn	8.45	3.54	0.62	0.74
14	hd1#	0.94	14	hn	8.45	3.49	0.54	0.76
15	ha	3.98	15	hb#	1.45	2.28	0.09	0.28
15	hb#	1.46	15	hn	7.92	2.57	0.2	0.22
15	ha	3.98	15	hn	7.91	2.66	0.33	0.27

16	hb2	2.71	16	hn	8.09	2.52	0.28	0.24
16	ha	4.46	16	hb1	2.79	2.93	0.41	0.33
16	hb1	2.8	16	hn	8.09	3.28	0.52	0.42
16	ha	4.45	16	hn	8.08	3.35	0.54	0.45
16	ha	4.46	16	hb2	2.72	3.03	0.56	0.49
17	ha	4.34	17	hb#	1.22	2.36	0.16	0.27
17	ha	4.33	17	hn	7.28	2.71	0.28	0.24
17	hb#	1.22	17	hn	7.28	3.02	0.34	0.29
18	ha1	0.66	18	hn	8.19	2.85	0.35	0.29
18	ha2	2.81	18	hn	8.18	2.33	0.3	0.29
19	hh2	7.35	19	hz2	7.58	2.19	0.18	0.59
19	hd1	7.57	19	he1	10.52	2.3	0.21	0.61
19	hz3	7.23	19	he3	7.83	2.43	0.25	0.63
19	ha	4.64	19	he3	7.84	2.7	0.3	0.45
19	hb2	3.51	19	he3	7.84	4.1	0.76	0.81
19	hb1	3.17	19	hd1	7.56	3.21	0.37	0.5
19	hb1	3.17	19	hn	8.75	3.62	0.41	0.33
19	ha	4.65	19	hb1	3.19	2.6	0.27	0.31
19	ha	4.64	19	hn	8.75	2.54	0.48	0.38
19	hb1	3.17	19	he3	7.84	3.19	0.49	0.6
19	ha	4.63	19	hb2	3.51	3.27	0.52	0.42
19	hd1	7.57	19	hn	8.75	3.46	0.58	0.68
19	hb2	3.52	19	hd1	7.56	2.69	0.59	0.69
19	hb2	3.52	19	hn	8.75	2.72	0.43	0.48
19	ha	4.64	19	hd1	7.56	4.23	0.82	1.05
1	hd#	3.18	1	he	7.42	2.41	0.25	0.22
1	ha	4.51	1	hb*	1.93	2.75	0.45	0.36
1	ha	4.51	1	hb*	1.96	2.7	0.5	0.4
1	hg2	1.65	1	he	7.41	3.51	0.6	0.5
1	hg1	1.82	1	he	7.41	3.56	0.61	0.52
1	hb*	1.93	1	he	7.41	3.95	0.73	0.69
1	ha	4.51	1	hg2	1.63	3.99	0.75	0.71
1	ha	4.51	1	hg1	1.81	4.25	0.83	0.86
20	ha	4.48	20	hb#	1.4	2.36	0.13	0.19
20	ha	4.49	20	hn	7.85	2.69	0.27	0.39
20	hb#	1.4	20	hn	7.84	2.82	0.27	0.26
21	ha	4.05	21	hb1	3.87	2.4	0.24	0.22
21	ha	4.05	21	hb2	3.46	2.73	0.35	0.28
21	ha	4.06	21	hn	7.58	2.85	0.38	0.31
21	hb2	3.48	21	hn	7.57	3.11	0.47	0.37
21	hb1	3.87	21	hn	7.57	3.19	0.65	0.57
2	hd1	3.82	2	hg2	2.11	3.01	0.44	0.35
2	hd1	3.82	2	hg1	2.01	2.77	0.49	0.39
2	ha	4.01	2	hb2	1.7	3.26	0.51	0.42
2	hd2	3.52	2	hg1	2.02	3.31	0.53	0.43
2	hd2	3.53	2	hb2	1.72	3.76	0.67	0.6
2	hd1	3.81	2	hb1	2.41	4.1	1	1.29
2	ha	4.01	3	hd1	3.24	2.51	0.31	0.26
2	ha	4.01	3	hd2	2.55	2.73	0.41	0.33
3	hd2	2.54	3	hg2	1.45	2.67	0.46	0.37
3	hd1	3.24	3	hg1	1.66	2.33	0.27	0.38
3	hd2	2.56	3	hg1	1.66	3.02	0.33	0.44
3	ha	2.27	3	hb2	1.11	2.93	0.26	0.39
3	ha	2.26	3	hb1	-0.09	2.3	0.47	0.4
3	hg2	1.42	3	hb1	-0.08	3.01	0.39	0.43
3	hg1	1.66	3	hb1	-0.05	2.28	0.32	0.47
4	ha	4.34	4	hb1	2.15	2.59	0.3	0.25
4	hd1	3.06	4	hg1	1.78	2.58	0.4	0.32

4	hd2	2.72	4	hg2	1.73	2.48	0.49	0.39
4	hd2	2.71	4	hg1	1.79	3.33	0.54	0.44
4	ha	4.34	4	hb2	1.95	3.1	0.56	0.46
4	hd1	3.04	4	hg2	1.71	3.41	0.56	0.47
4	hd1	3.06	4	hb1	2.15	3.57	0.61	0.52
4	hd2	2.71	4	hb2	1.95	4	0.75	0.71
4	hd2	2.71	4	hb1	2.15	4.18	0.81	0.82
5	ha	4.2	5	hb1	3.76	2.6	0.31	0.26
5	ha	4.19	5	hb2	3.83	2.85	0.32	0.37
5	ha	4.19	5	hn	8.21	2.69	0.33	0.27
5	hb1	3.77	5	hn	8.22	2.73	0.51	0.41
5	hb2	3.84	5	hn	8.22	3.21	0.53	0.43
6	ha	4.51	6	hn	7.85	2.88	0.38	0.33
6	hb2	2.62	6	hn	7.84	2.69	0.34	0.28
6	hb1	2.72	6	hn	7.84	2.72	0.35	0.28
6	ha	4.51	6	hb2	2.62	2.77	0.36	0.29
6	ha	4.52	6	hb1	2.71	2.55	0.23	0.21
8	ha	4.24	8	hb#	1.5	2.56	0.26	0.37
8	ha	4.24	8	hn	8.33	2.67	0.33	0.32
8	hb#	1.5	8	hn	8.33	2.4	0.14	0.2
9	ha	4.47	9	hb#	1.54	2.48	0.16	0.21
9	ha	4.47	9	hn	8.26	2.62	0.31	0.26
9	hb#	1.54	9	hn	8.26	2.74	0.25	0.24
i, i±1								
10	hn	8.53	11	hn	8.4	2.68	0.27	0.24
10	hb1	3.19	11	hn	8.41	2.96	0.42	0.33
10	hb2	3.12	11	hn	8.4	3.8	0.69	0.62
10	ha	3.94	11	hn	8.41	3.76	0.69	0.64
11	hn	8.43	10	hd#	7	3.65	0.62	0.94
11	ha	4.13	10	hd#	7	4.06	0.75	1.11
11	hb#	1.59	12	hn	8.15	3.12	0.37	0.31
11	ha	4.13	12	hn	8.15	3.55	0.61	0.51
12	hn	8.16	11	hn	8.41	2.89	0.4	0.32
12	hb1	2.1	13	hn	7.96	2.89	0.4	0.32
12	hb2	2.19	13	hn	7.96	3.26	0.52	0.42
13	hn	7.97	12	hn	8.15	2.47	0.27	0.23
13	hb1	3.52	14	hn	8.45	2.65	0.32	0.27
13	hn	7.97	14	hn	8.45	2.67	0.33	0.27
13	he3	6.82	14	hn	8.45	2.9	0.4	0.52
13	hb2	3	14	hn	8.45	3.61	0.63	0.54
14	hg	1.51	13	he3	6.81	2.72	0.41	0.52
14	ha	3.25	13	he3	6.81	3.37	0.55	0.65
14	hb2	1.73	13	he3	6.81	3.62	0.63	0.74
14	hd2#	0.88	13	he3	6.81	3.64	0.53	0.66
14	hd2#	0.88	13	hz3	6.87	2.72	0.56	0.68
14	hg	1.52	13	hz3	6.87	3.87	0.71	0.85
14	ha	3.25	13	hz3	6.87	3.93	0.73	0.88
14	hb1	1.44	13	he3	6.81	4.14	0.79	0.99
14	hd1#	0.94	13	hz3	6.87	4.32	0.75	0.88
14	hd1#	0.94	13	he3	6.81	4.62	0.84	1.01
14	hb1	1.44	15	hn	7.92	3.21	0.41	0.46
14	hb2	1.73	15	hn	7.92	2.82	0.37	0.3
14	ha	3.25	15	hn	7.92	3.52	0.6	0.5
15	hn	7.93	14	hn	8.45	2.64	0.32	0.27
15	hb#	1.47	16	hn	8.09	3.28	0.42	0.34
15	ha	3.98	16	hn	8.09	3.63	0.63	0.55

15	hn		16	hn		2.8	0.35	0.35
16	ha	4.46	17	hn	7.28	3.83	0.7	0.63
16	hb1	2.8	17	hn	7.31	3.99	0.75	0.71
16	hb2	2.72	17	hn	7.28	4.42	0.88	0.98
17	hn	7.29	16	hn	8.09	2.91	0.37	0.3
17	hn	7.29	18	hn	8.18	2.46	0.13	0.22
17	ha	4.34	18	hn	8.18	3.59	0.62	0.53
17	hb#	1.22	18	hn	8.18	4.26	0.73	0.62
18	ha2	2.81	17	hn	7.28	4.25	0.83	0.86
18	ha2	2.81	19	hn	8.75	3.1	0.57	0.38
18	ha1	0.65	19	hn	8.75	2.6	0.38	0.51
19	ha	4.63	18	ha2	2.81	4.07	0.77	0.76
19	ha	4.63	20	hn	7.84	3.1	0.39	0.45
1	ha	4.51	2	hd1	3.81	2.35	0.35	0.25
1	ha	4.51	2	hd2	3.52	2.86	0.39	0.31
1	hb*	1.97	2	hd2	3.52	3.89	0.4	0.42
1	hb*	1.93	2	hd2	3.52	3	0.53	0.43
20	hn		19	hn		2.82	0.27	0.26
20	ha	4.49	21	hn	7.57	3.39	0.56	0.46
20	hb#	1.4	21	hn	7.57	4.5	0.81	0.72
3	hd1	3.24	2	hb1	2.42	3.43	0.57	0.47
4	hd1	3.05	3	ha	2.26	2.35	0.22	0.33
4	hd2	2.71	3	ha	2.27	2.65	0.48	0.38
4	hd2	2.71	3	hb2	1.11	3.8	0.69	0.62
4	hd2	2.72	3	hb1	-0.07	4.03	0.76	0.73
4	hd1	3.05	3	hb2	1.12	4.13	0.79	0.79
4	ha	4.34	5	hn	8.22	2.25	0.2	0.05
4	hb2	1.95	5	hn	8.22	3.68	0.65	0.57
4	hb1	2.15	5	hn	8.22	4.39	0.87	0.95
5	ha	4.19	6	hn	7.85	3.45	0.57	0.48
5	hb1	3.77	6	hn	7.84	4.05	0.77	0.74
5	hb2	3.85	6	hn	7.84	4.17	0.8	0.81
6	hn	7.85	5	hn	8.22	2.68	0.46	0.39
6	ha	4.51	7	hn	8.76	2.68	0.51	0.48
6	hn	7.86	7	hn	8.75	4.35	0.46	0.4
8	hn	8.34	7	hn	8.76	2.59	0.52	0.62
8	hn	8.33	9	hn	8.26	2.54	0.19	0.4
8	hb#	1.5	9	hn	8.27	3.32	0.43	0.35
9	hn	8.27	10	hn	8.54	2.47	0.32	0.27
9	hb#	1.53	10	hn	8.53	3.55	0.5	0.4
9	ha	4.48	10	hn	8.54	3.48	0.61	0.57
i, i±n, n= 2-4								
10	ha	3.94	13	hb1	3.52	3.07	0.42	0.34
10	ha	3.94	13	hn	7.95	3.21	0.5	0.4
10	ha	3.94	13	he3	6.81	3.77	0.68	0.8
10	ha	3.93	13	hb2	3	3.48	0.68	0.61
10	ha	3.94	14	hn	8.45	3.93	0.73	0.68
11	ha	4.13	14	hn	8.45	3.24	0.45	0.35
11	ha	4.13	14	hb2	1.73	3.72	0.5	0.4
11	ha	4.13	14	hd1#	0.94	3.59	0.52	0.41
11	hb#	1.59	14	hn	8.45	4.86	0.82	0.74
12	ha	3.96	15	hb#	1.46	2.61	0.18	0.31
12	ha	3.96	15	hn	7.92	3.05	0.45	0.36
12	ha	3.96	16	hn	8.09	3.85	0.7	0.64
13	ha	4.12	16	hb2	2.71	3.17	0.49	0.39

13	ha	4.12	16	hb1	2.8	3.64	0.64	0.55
13	ha	4.12	16	hn	8.09	3.91	0.72	0.67
14	hd1#	0.94	10	he2	6.75	3.24	0.4	0.57
14	hd2#	0.87	10	he2	6.75	3.39	0.45	0.6
14	hg	1.52	10	he2	6.75	3.57	0.61	0.72
14	hg	1.51	10	hd2	7	3.84	0.7	0.64
14	hd1#	0.94	10	hd2	7	4.23	0.72	0.61
14	hd2#	0.87	10	hd2	7	4.79	0.9	0.86
14	hg	1.51	11	hn	8.4	4.59	0.7	0.69
14	ha	3.25	16	hn	8.09	4.05	0.76	0.74
14	ha	3.25	17	hn	7.28	3.65	0.64	0.55
14	ha	3.25	18	hn	8.18	2.98	0.43	0.34
14	ha	3.24	18	ha2	2.81	3.69	0.65	0.57
14	ha	3.25	18	ha1	0.65	4.23	0.82	0.85
15	hb#	1.46	12	he#	7.04	3.88	0.61	0.49
15	hb#	1.47	12	hn	8.16	4.63	0.85	0.78
15	ha	3.98	17	hn	7.28	3.93	0.73	0.68
16	hb2	2.71	18	hn	8.18	4.04	0.76	0.74
21	hb2	3.86	18	ha2	2.81	3.53	0.54	0.44
21	hb*	3.47	18	hn	8.18	3.66	0.64	0.56
21	hb1	3.46	18	ha2	2.8	4.02	0.76	0.73
4	hb2	1.95	6	hn	7.84	3.05	0.45	0.36
6	hb1	2.72	9	hb#	1.54	4.21	0.4	0.33
6	hb*		9	hb#		4.51	0.4	0.33
9	ha	4.48	12	hn	8.15	3.44	0.57	0.48
9	ha	4.47	12	hb*	2.1	3.81	0.69	0.62
9	ha	4.47	12	hb*	2.18	4.17	0.8	0.81
9	hb#	1.53	12	hn	8.15	5.22	1.03	1.11
9	hb#	1.53	6	hn	7.84	5.52	1.13	1.33
6	o		10	hn		2.25	0.25	0.25
7	o		11	hn		2.25	0.25	0.25
8	o		12	hn		2.25	0.25	0.25
3	hb1		5	hn		4.5	0.5	2.5
3	hb2		5	hn		4.5	0.5	1.5
i, i±n, n ≥ 5								
10	ha	3.94	4	hg1	1.78	3.05	0.51	0.42
10	ha		4	hg2		2.35	0.23	0.22
10	ha	3.94	4	hd2	2.71	2.78	0.55	0.45
13	hh2	6.53	19	hd1	7.56	3.62	0.63	0.94
13	hz2	6.1	19	hd1	7.56	3.69	0.65	0.97
13	hz2	6.1	19	he3	7.83	4.2	0.81	1.23
13	hh2	6.53	19	hn	8.73	4.46	0.89	1.2
13	hd1	6.76	1	he	7.4	3.63	0.63	0.75
13	hb2	3	4	hg1	1.78	3.45	0.58	0.48
18	ha1	0.66	13	hz3	6.87	4.07	0.77	0.96
19	ha	4.63	13	hz2	6.09	3.87	0.71	0.85
19	ha	4.63	13	he1	9.21	4.29	0.84	1.09
1	hg2	1.65	13	hd1	6.75	2.74	0.35	0.49
1	hg1	1.82	13	hd1	6.75	2.95	0.42	0.53
1	hd#	3.17	13	hd1	6.75	3.2	0.5	0.6
1	hg2	1.65	13	he1	9.21	3.37	0.55	0.65
1	hb*	1.97	13	hd1	6.75	3.78	0.68	0.81
1	hg1	1.81	13	he1	9.21	3.96	0.74	0.9
1	hb*	1.93	13	hd1	6.75	4.03	0.76	0.93
2	ha	4.03	13	hz2	6.1	3.33	0.53	0.68

2	ha	4.01	19	hz3	7.24	2.99	0.42	0.53
2	ha	4	19	he3	7.83	3.9	0.72	0.86
2	hb1	2.42	19	hz3	7.23	3.4	0.52	0.66
2	hb1	2.42	19	hh2	7.34	2.28	0.35	0.31
3	hb2	1.12	10	he1	6.75	3.17	0.49	0.59
3	hb1	-0.08	10	he1	6.75	3.5	0.59	0.7
3	hb2	1.12	10	hd1	7	3.5	0.59	0.7
3	hb1	-0.09	10	hd1	7	4.53	0.92	1.26
3	hd2	2.55	13	hz2	6.09	3.14	0.48	0.58
3	ha	2.27	13	he1	9.21	3.83	0.7	0.83
3	ha	2.27	13	hd1	6.75	3.9	0.72	0.87
3	hd2	2.53	13	hh2	6.52	4.27	0.84	1.08
3	hb1	-0.09	13	hz3	6.87	4.32	0.85	1.11
3	hb1	-0.08	13	hz2	6.09	4.42	0.88	1.18
3	hb1	-0.09	13	he3	6.8	4.69	0.97	1.38
3	hd1	3.23	19	hh2	7.34	3.72	0.66	0.79
3	hd1	3.23	19	hz3	7.24	3.91	0.72	0.87
4	hd2	2.71	10	hd1	7.01	3.08	0.39	0.49
4	hg2	1.73	10	hd1	7.01	3.5	0.65	0.57
4	hg2	1.71	10	hn	8.54	3.28	0.54	0.41
4	hb2	1.94	10	hn	8.54	4.12	0.79	0.78
4	hg1	1.79	10	hn	8.54	4.22	0.82	0.84
4	hg2		10	he1		5.22	0.82	0.84
4	hd1	3.05	13	hd1	6.75	3.01	0.44	0.55
1	o		13	he1		2.25	0.25	0.25
9	hb#		4	hd2		4.35	0.5	0.6
9	hb#		4	hb2		3.5	0.5	0.5
9	hb#		4	hg1		2.9	0.4	0.4

Structural Features of the Trp-cage and its Circular Permutants

The dense web of long range interactions in the Trp-cage motif includes the docking of P^{18}-P^{19} rings onto the Trp^{6} and Tyr^{3} aryl moieties of the helix, the close association of the G^{11}-CH_2 and the Trp^{6} indole ring, an Asp^{9}/Arg^{16} salt bridge [*12*], and a Trp^{6}-Hε1 to O=C-Arg^{16} H-bond. Most of these are reflected in both long-range NOEs and ring current shifts; however, these alone do not serve to define the Asp^{9}/Arg^{16} salt bridge. While the pH effect (3.1 kJ/mol of fold-destabilization on acidification) observed for cp-TC1 is comparable to that observed in Trp-cage constructs with a stabilizing Asp^{9}/Arg^{16} interaction [*5b*, *12*], we cannot rule out the possibility that the pH effect represents the removal of a fold-favoring Coulombic effect between the termini of the RPPPSGG-DAYAQWLADGGPSS sequence. However, the chemical shifts also support the presence of the salt bridge in the folded state. The downfield shift of Arg^{16} Hε in cp-TC1 goes from 0.38 (TFE added) to 0.20 (pH 7) to 0.05 (pH 2); while a downfield shift of the Arg^{16} Hγ reports on Arg^{16} sidechain placement near the plane of the Trp^{6} indole ring.

In the case of the circularly permuted cage with two Trp residues, both the NMR ensembles and the ring current shifts indicate an essentially identical orientation of the two Trps as well as the Gly, Tyr, two prolines and the Leu in the hydrophobic core. Minor differences in ring current shifts (Fig. 2S, panel B) at the Pro immediately adjacent to the added loop in the circular permutant are observed, indicating the loop closure does effect a small backbone conformational change. The same proline (P19 and P4) dihedral angles are observed in cyclo-TC1.

References – *all citation numbers [##] are those appearing in the communication text.*